Spindle checkpoint silencing requires association of PP1 to both Spc7 and kinesin-8 motors

Developmental Cell
Previews

Moving Right Along: How PP1 Helps Clear the Checkpoint

María Maldonado[1] and Tarun M. Kapoor[1,*]
[1]Laboratory of Chemistry and Cell Biology, The Rockefeller University, New York, NY 10065, USA
*Correspondence: kapoor@rockefeller.edu
DOI 10.1016/j.devcel.2011.05.017

Spindle checkpoint silencing is crucial for cell-cycle progression, but mechanisms underlying this process remain mysterious. Two papers, one in this issue of *Developmental Cell* (Meadows et al., 2011) and one in *Current Biology* (Rosenberg et al., 2011), begin to show how phosphatase PP1-gamma connects chromosome-microtubule attachment with anaphase entry.

Chromosome biorientation, the process by which sister chromatid kinetochores attach to microtubules emanating from opposite poles of the cell, is essential for error-free chromosome segregation. The spindle assembly checkpoint (SAC), a conserved surveillance mechanism in eukaryotic cells, prevents anaphase onset before all chromosomes are bioriented, thus ensuring the fidelity of cell division. Because even a single nonbioriented chromosome can delay anaphase onset, the SAC not only detects the primary error signal (the lack of biorientation) but also transduces it into a robust cytoplasmic "stop signal" that prevents chromosome segregation. Just as important as the ability to stop progression and correct errors is the ability to recognize that the checkpoint has been satisfied and move on; that is, once biorientation is achieved, the SAC must be "silenced" so that anaphase can follow.

In contrast to the numerous cell-biological, biochemical, and structural advances in our understanding of the establishment of checkpoint arrest, the elucidation of checkpoint silencing pathways has lagged behind. In human cells, at least three different processes for SAC silencing, which relieves the inhibition of cdc20 and allows the APC/C to ubiquitylate cyclin B and securin for entry into anaphase, have been suggested (Figure 1). These include motor-protein-based processes, such as the dynein-dependent removal of checkpoint proteins from microtubule-attached kinetochores (Howell et al., 2001) and the CENP-E-mediated silencing of BubR1 signaling (Mao et al., 2005). There are also mechanisms that involve inhibition of active SAC signaling proteins, such as the p31/Comet-mediated, structural mimicry-based inhibition of "active" Mad2 (Xia et al., 2004). Lastly, there are pathways that mediate the chemical modification of checkpoint proteins, such as the ubiquitylation of cdc20 (Reddy et al., 2007), or the dephosphorylation of "key" substrates of mitotic kinases by phosphatases. Dissection of this last mechanism has greatly benefited from studies in yeast, organisms in which, notably, most of the other silencing pathways are currently thought to be nonessential or nonexistent. Recent studies in budding and fission yeast have shown that the phosphatase PP1-gamma is essential for checkpoint silencing (Pinsky et al., 2009; Vanoosthuyse and Hardwick, 2009). These important discoveries laid the foundation for two new studies—one by Meadows and colleagues published in this issue of *Developmental Cell* (Meadows et al., 2011) and one by Rosenberg et al. published in *Current Biology* (Rosenberg et al., 2011)—that reveal how microtubule attachment is translated into SAC silencing by PP1-gamma.

The precise regulation of phosphorylation status is crucial in checkpoint signaling and silencing. In principle, one could regulate phosphorylation by controlling kinase levels or activity, modulating substrate specificity, regulating the subcellular localization, or controlling spatial separation of the kinase from its substrates. Another possibility for fine-tuning phosphorylation levels is, of course, by regulating the opposing phosphatase by analogous mechanisms. The PP1 family, together with PP2A phosphatases, is one of the major mediators of serine and threonine (Ser/Thr) dephosphorylation in the cell, likely accounting for over 90% of these events. Because the number of Ser/Thr kinases vastly exceeds the number of phosphatases in most organisms (~10 to 1 in humans), PP1 and PP2A phosphatases achieve specificity by binding to diverse regulatory subunits at different subcellular locations (Shi, 2009). Regulation may also be achieved by controlling phosphatase protein levels or catalysis, for example through methylation of the C terminus of the catalytic subunit of PP2A (Shi, 2009). Nevertheless, despite being crucial components of phosphoregulation, mechanisms for phosphatase regulation have remained less well characterized than their kinase counterparts, especially in the context of the spindle assembly checkpoint.

A recent study in vertebrate cells showed that the phosphatase PP1-gamma is recruited to kinetochores by binding to the kinetochore-resident protein Knl1 (also called Blinkin or CASC5), where it can oppose phosphorylation of the checkpoint kinase Aurora B (Liu et al., 2010). Following up on this result, Meadows and colleagues (2011) examined the relationship between Knl1 and PP1 in fission yeast. Using a combination of biochemistry and genetics, they confirmed that, as in vertebrates, the kinetochore protein Spc7 (homolog of Knl1) has two conserved PP1-gamma binding sites and that this interaction is essential for viability. To examine the contribution of the Spc7-PP1 interaction to SAC silencing, they turned to their previously described "chemical genetics" assay, in which Aurora kinase can be specifically and acutely inhibited to silence the checkpoint in the absence of microtubules (Vanoosthuyse and Hardwick, 2009). Using this assay, they found that the recruitment of PP1 by Spc7 is indeed involved in

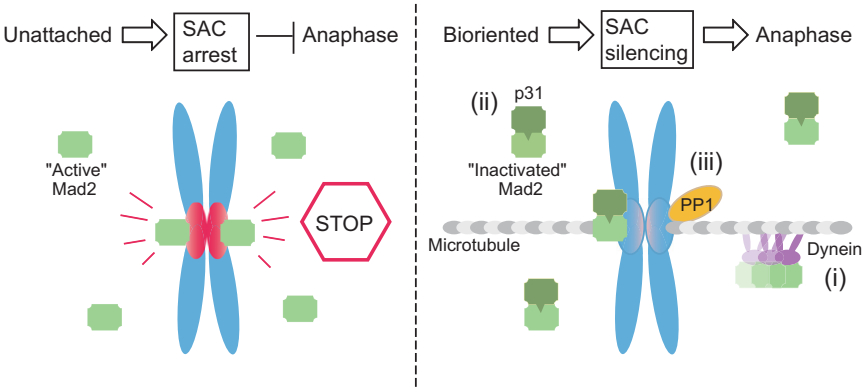

Figure 1. Mechanisms for Checkpoint Silencing

Left: Unattached kinetochores produce a "stop anaphase" signal involving the "active" form of the checkpoint protein Mad2, leading to checkpoint arrest. Kinetochores are highly phosphorylated (represented in red).

Right: Upon biorientation, the checkpoint may be silenced by several mechanisms, including (i) dynein-dependent removal of Mad2 from attached kinetochores, (ii) p31/Comet-mediated inhibition of kinetochore and cytoplasmic Mad2, and (iii) dephosphorylation of kinetochore and cytoplasmic substrates, for instance by the phosphatase PP1-gamma (PP1).

checkpoint silencing. Importantly, the independent study by Rosenberg et al. (2011) revealed that this Knl1-PP1 interaction is also crucial in budding yeast, in which the interaction between Spc105 (Knl1) and Glc7 (PP1) was found to be essential for viability due to its checkpoint silencing functions. Regardless of some differences in specific details, these findings confirm and clarify the roles of PP1-gamma in checkpoint silencing and suggest that this mechanism is conserved.

In addition, Meadows and coworkers (2011) found that abrogation of the Spc7-PP1 interaction did not completely abolish checkpoint silencing, suggesting that additional PP1-dependent mechanisms might be involved. When the authors looked for additional binding partners of PP1-gamma, they found that the kinesin motor proteins klp5 and klp6 interacted with the phosphatase in a way that was instrumental for timely checkpoint silencing. Interestingly, the motor domains of these kinesin proteins, which are known to be involved in the congression of chromosomes to the middle of the spindle, were found to be dispensable for their SAC silencing roles. Therefore, whether this mechanism is analogous to the motor-based silencing mechanisms seen in human cells, where motor proteins remove checkpoint proteins from microtubule-attached kinetochores, remains to be fully resolved. There are insufficient data upon which to speculate how these kinesins may be modulating PP1 activity in the absence of their motor activity; nevertheless, these results do show that several pools of PP1, differentially localized by its binding partners, are necessary for the global regulation of mitotic phosphorylation levels to allow entry into anaphase.

What is more, Rosenberg and colleagues (2011) further showed that it is not just PP1 localization that is important for its silencing roles; its local concentration at the kinetochore is also critical. Expression of either a Spc105 (Knl1) mutant that cannot recruit Glc7 (PP1) or a Spc105-Glc7 fusion protein is detrimental for viability. Notably however, expression of a fusion of Glc7 to a Spc105 mutant that cannot recruit endogenous Glc7 is viable. This suggests that even a 2-fold increase in the amount of PP1-gamma recruited by Knl1 leads to death. Together with the above findings, these results nicely illustrate how fine-tuning of phosphatase activity is as important as regulation of its opposing kinase(s) for the maintenance of appropriate levels of phosphorylation and, hence, viability.

These studies will no doubt motivate additional analyses of how PP1-gamma connects biorientation with checkpoint silencing. In particular, finding out what the relevant PP1 substrates are is now a key question and will likely be a major endeavor. It will also be interesting to determine the biochemical differences between the kinetochore-associated and cytoplasmic PP1-gamma pools and to determine how microtubule attachment regulates the dephosphorylation reaction.

We anticipate research will also be busy examining the interplay of PP1-gamma with the other silencing pathways implicated in anaphase entry in higher eukaryotes. Does PP1-mediated dephosphorylation affect motor-based silencing? Could PP1-gamma be responsible for activating the Mad2 inhibitor p31/Comet? Furthermore, a comparative analysis of silencing mechanisms across eukaryotes will be useful for inspecting how and when silencing complexity arose and whether it is correlated with the rise of complexity in kinetochore structure, attachment modes, and centromere specification. Exciting times lie ahead as we move along in the study of checkpoint silencing.

REFERENCES

Howell, B.J., McEwen, B.F., Canman, J.C., Hoffman, D.B., Farrar, E.M., Rieder, C.L., and Salmon, E.D. (2001). J. Cell Biol. *155*, 1159–1172.

Liu, D., Vleugel, M., Backer, C.B., Hori, T., Fukagawa, T., Cheeseman, I.M., and Lampson, M.A. (2010). J. Cell Biol. *188*, 809–820.

Mao, Y., Desai, A., and Cleveland, D.W. (2005). J. Cell Biol. *170*, 873–880.

Meadows, J.C., Shepperd, L.A., Vanoosthuyse, V., Lancaster, T.C., Sochaj, A.M., Buttrick, G.J., Hardwick, K.G., and Millar, J. (2011). Dev Cell *20*. 10.1016/j.devcel.2011.05.008, this issue, 739–750.

Pinsky, B.A., Nelson, C.R., and Biggins, S. (2009). Curr. Biol. *19*, 1182–1187.

Reddy, S.K., Rape, M., Margansky, W.A., and Kirschner, M.W. (2007). Nature *446*, 921–925.

Rosenberg, J.S., Cross, F.R., and Funabiki, H. (2011). Curr. Biol. *21*, 942–947.

Shi, Y.G. (2009). Cell *139*, 468–484.

Vanoosthuyse, V., and Hardwick, K.G. (2009). Curr. Biol. *19*, 1176–1181.

Xia, G., Luo, X., Habu, T., Rizo, J., Matsumoto, T., and Yu, H. (2004). EMBO J. *23*, 3133–3143.